U.S. National Institute of Health

TREASURY DEPARTMENT.

Public Health and Marine-Hospital Service of the United States.

WALTER WYMAN, Surgeon-General.

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Biological
Medical
Serials

HYGIENIC LABORATORY.—BULLETIN No. 12.

March, 1903.

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THE BACTERIOLOGICAL IMPURITIES OF VACCINE VIRUS.

Biological
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AN EXPERIMENTAL STUDY.

BY

M. J. ROSENAU,

DIRECTOR OF HYGIENIC LABORATORY.

WASHINGTON:
GOVERNMENT PRINTING OFFICE.
1903.

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The following *bulletins* [Bull. Nos. 1-7, 1900 to 1902, Hyg. Lab., U. S. Mar.-Hosp. Serv., Wash.] have been issued:

No. 1.—Preliminary note on the viability of the *Bacillus pestis.* By M. J. Rosenau.

No. 2.—Formalin disinfection of baggage without apparatus. By M. J. Rosenau.

No. 3.—Sulphur dioxide as a germicidal agent. By H. D. Geddings.

No. 4.—Viability of the *Bacillus pestis.* By M. J. Rosenau.

No. 5.—An investigation of a pathogenic microbe (*B. typhi murium* Danyz) applied to the destruction of rats. By M. J. Rosenau.

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No. 7.—Laboratory technique: Ring test for indol, by S. B. Grubbs and Edward Francis; Collodium sacs, by S. B. Grubbs and Edward Francis; Microphotography with simple apparatus, by H. B. Parker.

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Since the change of name of the service the bulletins of the Hygienic Laboratory have been continued in the same numerical order, as follows:

No. 8.—Laboratory course in bacteriology and pathology. By M. J. Rosenau.

No. 9.—Presence of tetanus in commercial gelatin. By John F. Anderson.

No. 10.—Report upon the prevalence and geographic distribution of hookworm disease (uncinariasis or anchylostomiasis) in the United States. By Ch. Wardell Stiles.

No. 11.—Experimental investigation of *Trypanosoma Lewisi.* By Edward Francis.

No. 12.—The bacteriological impurities of vaccine virus; an experimental study. By M. J. Rosenau.

In citing these bulletins, beginning with No. 8, bibliographers and authors are requested to adopt the following abbreviations: Bull. No. ——, Hyg. Lab., U. S. Pub. Health & Mar.-Hosp. Serv., Wash., pp. ——.

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THE BACTERIOLOGICAL IMPURITIES OF VACCINE VIRUS. AN EXPERIMENTAL STUDY.

(By MILTON J. ROSENAU, Director of the Hygienic Laboratory, Passed Assistant Surgeon, U. S. Public Health and Marine-Hospital Service.)

OBJECT OF THE WORK.

The widespread prevalence of smallpox in our country during the past few years gave abundant opportunity to corroborate the great prophylactic value of vaccination. Hundreds of thousands of vaccinations have been performed during this period with very satisfactory and beneficial results. However, we were not surprised to hear of some accidents and complications. There were sore arms and a few fatalities resulting from wound complications.

The question immediately arose whether the cause of these complications was contained in the vaccinal matter or whether it resulted from subsequent contamination of the wound from outside sources.

Clinical studies can not be depended upon to settle this question, because infected "takes" may result from other causes than a contamination of the virus.

To determine this question, therefore, we made bacteriological studies of the number and kinds of micro-organisms that contaminate the different makes of vaccine virus found on the open market. In our studies special attention was given to the pus cocci and to tetanus.

We were rather surprised in visiting some of the vaccine farms to find that the operators permitted a certain degree of carelessness in preparing and collecting material for the glycerinated virus, trusting, as they stated, "to the glycerin to take out the impurities." This opinion of the exalted power of glycerin we have shown to be based upon error. Glycerin will destroy a certain number of bacterial impurities, but it is impotent against gross contamination or spores. Our studies of the germicidal power of glycerin will be made the subject of another paper.

All the vaccines examined were purchased in the open market from reliable pharmacists, who keep the virus under suitable conditions of light and temperature. Our results therefore represent the impurities found in the vaccine virus sold to the practitioner.

INEVITABLE IMPURITIES IN BOVINE VACCINE VIRUS.

Vaccinia is a specific disease, the cause of which has not been determined. We are, therefore, working somewhat in the dark. We are compelled to vaccinate our patients with a virus containing micro-organisms other than those causing vaccinia. The importance of using a virus as pure as possible need not be emphasized here, for we do not want to inoculate our patients with any other infection than the one which protects the individual against smallpox. On account of this danger human virus has been discarded in many countries, despite the fact that human virus is believed to be superior to bovine virus so far as the reliability and the duration of its immunizing power are concerned.

The production of bovine virus by propagating it from heifer to heifer is credited to Negri, of Naples, about 1842. It took some years for the advantages of this virus to be appreciated. Practically no other kind is now used in the large communities of Europe and in our own country. The great advantage of bovine virus, in addition to the ease with which it may be procured, is that it absolutely eliminates the possibility of the transmission of syphilis and other infections peculiar to the human family.

Now, although bovine virus is free from the danger of conveying the infectious diseases peculiar to man, it is liable to other equally undesirable contaminations. For instance, in addition to the micro-organisms that are specific for vaccinia, it contains the pus cocci and the bacteria that live normally upon and in the skin of the calf, and these micro-organisms always contaminate bovine virus. It is impossible to use germicidal agents in the treatment of the vaccinal eruption of calves, as such substances would kill the potency of the virus. Therefore, it is evident that even the greatest care will not insure such virus against "foreign" organisms.

OBJECT OF GLYCERINATING THE VACCINE PULP.

In order to eliminate the danger of the contaminating bacteria, Dr. Moncton Copeman, in 1891, devised the method of mixing the pulp with sterile glycerin of first quality. The advantages which Copeman claimed for the glycerin were that it not only prevented the growth and multiplication of the bacteria, always found in bovine virus, but gradually destroyed those which were present.

Glycerin can hardly be dignified with a place among the germicides, although the object of adding it to vaccine virus is to destroy the contaminating micro-organisms. Bacteria are slowly killed by glycerin, just as they are killed by drying; the glycerin is supposed to produce its bactericidal effects by a process of slow dehydration. Germs with thick envelopes resist it indefinitely. It has no action upon endogenous spores at all; in fact, it is a preservative of such infections as tetanus, malignant edema, anthrax, and the like. In 80 and 90 per cent glycerin, we have kept as common and as easily destroyed an organism as the golden pus coccus alive forty-one days; the colon bacillus lived seventy days in 60 per cent glycerin; anthrax spores remained alive and virulent two hundred and seventy-eight days in glycerin. This work will soon be made the subject of another report.

Glycerin, therefore, can not take the place of care and cleanliness in the preparation of vaccine virus.

The effect of mixing glycerin with the virus is to gradually destroy both the bacteria and the vaccine, but fortunately the ordinary pus cocci and nonsporulating bacteria generally succumb before the viability of the vaccine organism is destroyed, and therefore there is an interval when the glycerinated virus will still cause a typical "take," but will contain comparatively few foreign micro-organisms. It is evident that if the glycerinated virus is used before this interval it has no advantage over the dry point, and if used after this interval it is inert. Therefore, from a theoretical view point glycerinated virus should be freer from impurities if used just at the right time. Manufacturers state that they usually glycerinate the virus from four to six weeks before putting it on the market.

The dry points, on the contrary, may be sold as soon as made and, if kept in a cool place protected from the light, they probably remain viable a longer time than the glycerinated virus under similar conditions. It is well known that pus cocci and the other bacteria which frequently contaminate vaccine virus die quickly when dry. These same bacteria, however, live a comparatively long time in dry vaccine virus, probably on account of the protection of the albuminous matter in which they are imbedded.

The glycerinated virus is made from the "pulp" that is scraped from the site of the eruption. This pulp consists of the inflammatory exudate and macerated cuticle. The mass is intimately ground with 50 to 60 per cent of glycerin, then placed aside in a cool place for several weeks until the pyogenic cocci and other pathogenic organisms disappear.

Until recently the dry points were always prepared by using the serum or "lymph" which exudes from the site of the eruption, after the crusts and epithelial layers have for the most part been removed. The sterile ivory points are then coated with this serum by means of a brush or the points are dipped directly into the exudate. This lymph is largely blood serum, mixed with inflammatory exudate. At present the dry points are mostly prepared from the glycerinated pulp. That is to say, the glycerinated virus, after it has stood a sufficient length of time to free itself of bacterial impurities, is dried upon ivory points. Sometimes the glycerinated virus is mixed with sterile blood serum in order to facilitate its drying and adhesion to the ivory point.

SEASONAL VARIATION IN THE PURITY OF VACCINE VIRUS.

A glance at the following tables will show the unevenness of the product of some manufactures. It will be noted that the contaminating microbes seemed to rise in numbers during the winter of 1901–2, when the demand for vaccine virus was very great, indicating perhaps haste in its production, or the marketing of an unripe product. It will also be noted that there is a marked improvement in the glycerinated product this winter (1902) as compared with that placed on the market last year. We believe that our warning, showing the feeble germicidal properties of glycerin, and an exposition of the fact that some of the glycerinated virus found upon the market was "green," helped materially toward this beneficial result. (See article in American Medicine, April 19, 1902, page 637, "Dry points versus glycerinated virus from a bacteriologic standpoint," read at a meeting of the New York Academy of Medicine, February 20, 1902, by M. J. Rosenau.)

Tables comparing the number of bacteria in the dry points and glycerinated virus marketed by the various manufacturers at different times.

MANUFACTURER NO. 1.

Dry points.			Glycerinated virus.		
Winter, 1901–2.	April and May, 1902.	November and December, 1902.	Winter, 1901–2.	April and May, 1902.	November and December, 1902.
4,923	7,240	0	192	22	1
6,240	7,326	0	1,332	24	2
9,050	7,625	1	1,456	28	2
9,289	7,625	1	6,249	46	2
9,884	9,430	2	6,876	72	3
10,629	10,050	3	7,274	101	3
14,826	10,340	3		—	4
20,828	10,345	3		48	4
	12,075	5		80	5
	12,100	11		102	9
				110	
				112	
				194	

MANUFACTURER NO. 3.

847	2,518	7,465	111	3,011	41
906	2,727	8,702	138	4,309	70
2,088	3,275	10,022	246	6,022	87
2,750	3,866	10,265	352	6,670	95
6,528	4,359	10,982	1,121	7,294	112
13,030	4,446	12,923	1,750	7,427	152
	5,577	15,552	2,070	9,309	230
	8,048	25,574	2,263	11,169	234
	8,384	26,411	2,440		239
	19,107	31,380			

Tables comparing the number of bacteria in the dry points, etc.—Continued.

MANUFACTURER NO. 4.

Dry points.			Glycerinated virus.		
Winter, 1901–2.	April and May, 1902.	November and December, 1902.	Winter, 1901–2.	April and May, 1902.	November and December, 1902.
1,530	636	243	1,414	24	3
2,160	1,998	262	1,540	54	4
2,376	2,069	420	1,842	68	6
8,024	2,433	424	2,928	84	11
9,688	2,726	452	10,372	97	16
12,800	2,780	465	11,232	144	19
	3,448	475	18,404	153	31
	3,960	479		165	37
	5,402	526		259	77
	8,160	576		393	113

MANUFACTURER NO. 5.

13	1	604	84	6	2
18	5	810	86	24	2
20	6	1,088	97	28	2
27	7	1,156	127	55	2
110	9	1,199	160	105	2
182	10	1,204	170		3
219	10	1,399	245		3
220	10	1,452	257		4
297	50	2,246	768		5
458		2,584	1,680		6
575			1,700		
648			2,069		
7,200			10,400		
11,200			17,000		
15,760					

MANUFACTURER NO 6.

None examined.			369	21	0
			747	694	0
			1,592	1,877	0
			1,912	2,368	1
			2,106	2,689	2
			2,578	3,326	2
			3,819	4,208	4
				4,352	4
				8,094	5
					11

Tables comparing the number of bacteria in the dry points, etc.—Continued.

MANUFACTURER NO. 7.

Dry points.			Glycerinated virus.		
Winter 1901-2.	April and May, 1902.	November and December, 1902.	Winter 1901-2.	April and May, 1902.	November and December, 1902.
	63	3	116	28	4
	88	3	160	32	4
	95	5	1,600	35	5
	106	5	2,400	35	5
	120	6	8,000	37	6
	125	7		44	6
	128	21		47	7
	164	31		51	7
	342	48		59	9
	380	67		101	9

MANUFACTURER NO. 8.

450	2,548	853	2,100	13	8
476	3,405	868	2,200	14	10
516	3,805	887		20	11
3,325	3,829	1,800		21	12
3,475	4,026	1,847		21	13
3,600	4,321	1,946		21	14
	4,445	2,074		26	16
	4,665	2,244		28	16
	5,121	2,912		31	19
	5,355	3,754		42	20

MANUFACTURER NO. 9.

	195	5,952	11,840	12	7
	245	7,632	12,350	32	10
	312	7,560	12,960	116	13
	440	7,980	15,680	214	25
	496	8,748	16,480	236	43
	576	8,748	17,680	345	47
	578	9,744	17,920	370	70
	665	11,020	23,800	415	81
	805	12,972	28,640	416	85
	1,000	19,872	30,080	541	105

MANUFACTURER NO. 10.

None examined.			0	0	7
			0	0	14
			0	3	15
			0	3	17
			0	3	21
			0	4	22
			0	4	22
			0	5	31
			3	7	31
			4	9	53

TECHNIQUE EMPLOYED IN COUNTING BACTERIA IN VACCINE VIRUS.

The virus was suspended in a measured quantity of sterile bouillon and agitated so that all the clumps were broken up and a uniform suspension obtained as nearly as possible. The dry points were first softened in a small quantity of bouillon and then rubbed clean, always using the usual bacteriological precautions to prevent contamination from the outside. The glycerinated virus was mixed with a small quantity of bouillon, and the capillary tube washed out by drawing the liquid in and out of the tube a number of times.

The mixing was done in test glasses of appropriate size and the mixture thoroughly agitated. It is very important to agitate the suspension thoroughly in order to break up the clumps. This may best be done by drawing the fluid into pipettes and blowing it out vigorously, repeating the operation a number of times.

This suspension was now planted in agar and plated on Petri dishes. No less than three plates were made of each point or capillary tube; one or two drops of the suspension being planted in the first plate, five or ten drops in the second, and the total quantity remaining in the third plate. In this way the figures gave an accurate count of all the colonies that grew from each vaccine examined, excepting in those instances where the vaccine contained excessive numbers of bacteria. In such cases it was necessary to estimate the total number from the first or second plate.

The plates were grown in the incubator at 37° C., and the counts made when the maximum growth appeared, usually upon the third day.

It is practically impossible to determine the absolute number of organisms in a given virus, for it consists of an inflammatory product which is very variable in its physical characteristics. Upon dry points it coagulates into a hard, insoluble film; and mixed with glycerin it always contains little masses and agglutinated flakes and particles which hold enmeshed the micro-organisms. It is practically impossible to ultimately break up these masses. Therefore, the suspensions are not uniform and the counts we make are only approximations. Micro-organisms have a well-known tendency to group or cling together, so that every colony upon an agar plate does not represent the growth from one single microbe. The figures as given, therefore, are misleading only in that they give an underestimate of the number of organisms contaminating vaccine virus, and therefore some of the results, as bad as they are, do not fully represent the actual conditions.

The capillary tubes containing glycerinated virus vary considerably in capacity; some hold three and four times as much as others. This fact partly explains the discordance in some of the figures, but it is not sufficient to justify the marked discrepancy which we have found existing between tubes from the same package and bearing the same laboratory number as given by the manufacturer.

The following tables give the results of our counts, classified according to the manufacturer. They are given in three series: First, the result of our work during the winter of 1901–2; second, the result of our work during the summer (April and May); and, third, in November and December, 1902. We were surprised to find such a large number of colonies, especially from the glycerinated virus, and therefore repeated our work very carefully. The counts were made by Dr. Grubbs, Dr. Parker, Dr. Francis, and Dr. Anderson.

NUMBER OF BACTERIA IN VACCINE VIRUS BOUGHT ON THE OPEN MARKET, WITH A SPECIAL STUDY OF THE PYOGENIC COCCI.

The number of bacteria found in vaccine virus is of course not so important a factor as the kind of organisms. A few tetanus spores or virulent pus cocci would be of far more serious moment than myriads of the common saprophytes. However, the number of organisms found to contaminate vaccine virus is a fair index of its purity, as well as of the care taken in its production. The number of bacteria found in glycerinated virus is also an indication of its "ripeness."

We counted 190 dry points and 244 glycerinated tubes. The dry points averaged 4,354 bacteria per point and the tubes 1,742 per tube.

Some points contained as great a number as 44,000 foreign organisms. The highest count found in the capillary tubes containing glycerinated virus was 30,080. A few (very few) points and tubes were sterile, i. e., gave no bacterial growth either on agar plates or in bouillon.

SERIES 1.

The following tables give the results of our studies during the winter of 1901–2:

MANUFACTURER NO. 1.

Number of organisms found.	
Dry points.	Glycerinated virus.
[a] 9,289	[b] 192
[a] 14,826	[b] 1,332
[a] 20,828	[b] 1,456
[c]	[b] 6,249
6,240	[b] 6,876
9,884	[b] 7,274
[c] ———	
4,923	
9,050	
10,629	

[a] Orange, yellow, and white staphylococci were isolated from these points; not pathogenic for mice, rats, and guinea pigs.

[b] Yellow and white staphylococci were isolated from these tubes. They produced no effect upon mice, rats, and guinea pigs.

[c] These short lines separate samples bearing a different number as given by the manufacturer.

This virus evidently contains more bacteria than a good virus should. The ordinary cocci of suppuration were found in both the dry points and the glycerinated virus; and although these organisms when inoculated into laboratory animals gave no results, there is little indication that under favorable circumstances they might not be pathogenic for man. While the glycerin has reduced the average number of bacteria found in this virus, it still has pathogenic bacteria that it should not contain; and, so far as the numbers are concerned, the samples examined, with one exception (192), are far above the number allowed a good virus.

MANUFACTURER NO. 3.

Number of organisms found.	
Dry points.	Glycerinated virus.
847	2,263
906	———
2,088	1,750
———	2,070
2,750	2,440
6,528	———
13,030	111
	138
	246
	352
	1,121

A great many molds common to the air and to hay, were found in this virus, indicating stable contamination.

MANUFACTURER NO. 4.

Number of organisms found.	
Dry points.	Glycerinated virus.
1,530	10,372
2,376	11,232
2,160	18,404
8,024	1,414
9,688	1,540
12,800	1,842
	2,928

Here we have a manufacturer who puts up both kinds of virus, but his glycerinated product contains more bacteria than his dry points.

MANUFACTURER NO. 5.

Number of organisms found.	
Dry points.	Glycerinated virus.
110	84
182	768
220	1,680
575	1,700
7,200	2,069
11,200	10,400
15,760	17,000
[a]219	[a]86
[a]297	[a]97
[a]458	[a]160
[a]648	[a]257
[b]13	[b]127
[b]18	[b]170
[b]20	[b]245
[b]27	

[a] Taken from the same lot and examined three months later.
[b] Another sample.

The only comment that it seems necessary to make on the product of this manufacturer is its unevenness. While the average is fair, we found two capillary tubes of glycerinated virus to contain an excessive contamination. It may be that these tubes were placed upon the market before they were sufficiently glycerinated.

MANUFACTURER NO. 6.

Number of organisms found.	
Dry points.	Glycerinated virus.
None examined.	369
	747
	2,106
	1,592
	1,912
	2,578
	3,819

MANUFACTURER NO. 7.

Number of organisms found.	
Dry points.	Glycerinated virus.
None examined.	116
	160
	1,600
	2,400
	8,000

MANUFACTURER NO. 8.

Number of organisms found.	
Dry points.	Glycerinated virus.
450	[a] 2,100
476	[a] 2,200
516	
[b] —	
3,325	
3,475	
3,600	

[a] The ordinary pus cocci (white, yellow, and orange) were isolated from this virus, but when inoculated into animals gave negative result.

[b] This line separates samples bearing a different number as given by the manufacturer.

Nine weeks later other samples of the glycerinated virus out of the same box were counted and gave only 30 bacteria per tube. This would seem to indicate that the virus was "green" when placed upon the market, and that if the manufacturer had kept it a few weeks longer it would have been freer from contamination.

MANUFACTURER NO. 9.

Number of organisms found.	
Dry points.	Glycerinated virus.
None examined.	11,840
	12,350
	12,960
	15,680
	16,480
	17,680
	17,920
	23,800
	28,640
	30,080

MANUFACTURER NO. 10.

Number of organisms found.	
Dry points.	Glycerinated virus.
None examined.	0
	0
	0
	0
	0
	0
	0
	0
	3
	4

SUMMARY OF SERIES I.

Winter 1901–2.

Number of organisms found.		
Dry points.	Glycerinated tubes.	
13	0	2,100
18	0	2,106
20	0	2,200
27	0	2,263
110	0	2,400
182	0	2,440
219	0	2,578
220	0	2,928
297	3	3,819
450	4	6,249
458	30	6,876
476	84	7,249
516	86	8,000
575	97	10,372
648	111	10,400
847	116	11,232
906	127	11,840
1,530	138	12,350
2,088	160	12,960
2,160	160	15,680
2,376	170	16,480
2,750	192	17,000
3,325	245	17,680
3,475	246	17,920
3,600	257	18,404
4,923	352	23,800
6,240	369	28,640
6,528	747	30,080
7,200	768	
8,024	1,121	
9,050	1,332	
9,289	1,414	
9,688	1,456	
9,884	1,540	
10,629	1,592	
11,200	1,600	
12,800	1,680	
13,030	1,700	
14,826	1,750	
15,760	1,842	
20,828	1,912	
	2,069	
	2,070	

Number points examined	41	Number tubes examined	71
Total bacteria	197,185	Total bacteria	333,586
Average per point	4,809	Average per tube	4,698

SERIES II.

The following tables give the results of our studies during the spring of 1902:

MANUFACTURER NO. 1.

Number of organisms found.	
Dry points.	Glycerinated virus.
7,240	22
7,326	24
7,625	28
7,625	46
9,430	72
10,050	101
10,340	48
10,345	80
12,075	102
12,100	110
	112
	194

The dry points were dated "to be exchanged after June 3, 1902," and were examined May 23, 1902. The plates contained many colonies of a yellow staphylococcus resembling pus organisms.

The glycerinated virus was dated by the maker "to be exchanged after April 25, 1902," and was examined April 26, 1902.

MANUFACTURER NO. 3.

Number of organisms found.	
Dry points.	Glycerinated virus.
2,518	3,011
2,727	4,309
3,275	6,022
3,866	6,670
4,359	7,294
4,446	7,427
5,577	9,309
8,048	11,169
8,384	
19,107	

The dry points were guaranteed by the manufacturer until June 7, 1902, and were examined April 30, 1902.

The glycerinated virus was guaranteed by the manufacturer until June 17, 1902, and was counted April 30, 1902.

MANUFACTURER NO. 4.

Number of organisms found.	
Dry points.	Glycerinated virus.
636	24
1,998	54
2,069	68
2,433	84
2,726	97
2,780	144
3,448	153
3,960	165
5,402	259
8,160	393

The dry points were labeled by the maker to be returned after May 28, 1902, and were counted May 14, 1902.

The glycerinated virus was labeled by the maker to be returned after June 17, 1902, and was examined May 15, 1902.

MANUFACTURER NO. 5.

Number of organisms found.	
Dry points.	Glycerinated virus.
1	6
5	24
6	28
7	55
9	105
10	
10	
10	
50	

The dry points were labeled by the maker to be returned after May 18, 1902, and were planted April 25, 1902.

The glycerinated virus was labeled to be returned after May 11, 1902, and was examined April 25, 1902.

MANUFACTURER NO. 6.

Number of organisms found.	
Dry points.	Glycerinated virus.
None examined.	21
	694
	1,877
	2,368
	2,689
	3,326
	4,208
	4,352
	8,094

The glycerinated virus was stated by the manufacturer to be good until June 4, 1902, and was examined April 30, 1902.

Many yellow and white colonies grew on the plates from this vaccine resembling pus cocci. The following table shows the result of our study of 12 of these organisms:

Table of staphylococci isolated from

No.	Grouping.	Relation to Grams.	Indol.	Growth in milk.
^{3}10	Staphylococci	Stains	Negative	Very slight acid product.
^{3}10	do	do	do	No change
^{4}5	do	do	do	Light yellow
^{4}10	do	do	do	Light brown
^{4}10	do	do	do	Light yellow
^{5}10	do	Negative	do	Acid production; precipitate of casein.
^{6}10	do	Stains	do	No change
^{7}23	do	do	do	do
^{8}5	do	do	do	do
^{9}20	do	do	Very slight	Alkaline, brownish yellow precipitate.
^{10}65	do	do	Negative	No change
^{10}65	do	do	do	Dirty light blue, with scum on surface.

glycerinated virus of manufacturer No. 6.

Growth in gelatin.	Growth in bouillon.	Growth on potato.	Growth in glucose bouillon.	Growth in lactose bouillon.
No liquefaction	Diffused cloudiness.	White granular growth; scanty.	No fermentation; cloudy all through.	No fermentation; cloudy at bend of tube.
Slow liquefaction.	Slightly cloudy	Heavy growth; yellowish green.	No fermentation; slightly cloudy at bend of tube.	Do.
No liquefaction	do	Slight yellow growth.	No fermentation; cloudy at bend of tube.	Do.
do	do	No growth	No fermentation; very slight growth at bend of tube.	No fermentation; very slight growth at bend of tube.
do	do	Heavy yellow growth all over potato.	No fermentation; precipitate at bend of tube.	No fermentation; precipitate at bend of tube.
do	Cloudy	Heavy white growth spreading.	No fermentation; slightly cloudy at bend of tube.	No fermentation; slightly cloudy at bend of tube.
Very slow liquefaction.	do	Yellowish white growth; very heavy.	No fermentation; very slight growth at bend of tube.	No fermentation; very slight growth at bend of tube.
No liquefaction	do	Invisible growth	do	Do.
do	do	do	do	Do.
do	do	Abundant white growth, slowly spreading.	do	Do.
Slow liquefaction.	Slightly cloudy	Slow growth, yellow granular.	do	Do.
No liquefaction	Slightly cloudy; some precipitate.	No growth	do	Do.

MANUFACTURER NO. 7.

Number of organisms found.	
Dry points.	Glycerinated virus.
63	28
88	32
95	35
106	35
120	37
125	44
128	47
164	51
342	59
380	101

The dry points were stated by the maker to be good until June 15, 1902, and were examined April 26, 1902.

The glycerinated virus was labeled by the manufacturer to be good until May 1, 1902, and was counted April 26, 1902.

MANUFACTURER NO. 8.

Number of organisms found.	
Dry points.	Glycerinated virus.
2,548	13
3,405	14
3,805	20
3,829	21
4,026	21
4,321	21
4,445	26
4,665	28
5,121	31
5,355	42

Counted May 15, 1902. The packages contained neither date nor laboratory number.

MANUFACTURER NO. 9.

Number of organisms found.	
Dry points.	Glycerinated virus.
195	12
245	32
312	116
440	214
496	236
576	345
578	370
665	415
805	416
1,000	541

The dry points were prepared, according to the maker's label, May 1, 1902, and were counted May 15, 1902.

The glycerinated virus was not to be used after July 1, 1902, according to the maker's statement, and was examined May 15, 1902.

MANUFACTURER NO. 10.

Number of organisms found.	
Dry points.	Glycerinated virus.
None examined	0
	0
	3
	3
	3
	4
	4
	5
	7
	9

The glycerinated virus was marked to be returned after August 1, 1902, and was counted May 16, 1902.

SUMMARY OF SERIES II.

April and May, 1902.

Number of organisms found.			
Dry points.		Glycerinated tubes.	
1	5,355	0	110
5	5,402	0	112
6	5,577	3	116
7	7,240	3	144
9	7,326	3	153
10	7,625	4	165
10	7,625	4	194
10	8,048	5	214
50	8,160	6	236
63	8,384	7	259
88	9,430	9	345
95	10,050	12	370
106	10,340	13	393
120	10,345	14	415
125	12,075	20	416
128	12,100	21	541
164	19,107	21	694
195		21	1,877
245		21	2,368
312		22	2,689
342		24	3,011
380		24	3,326
440		24	4,208
496		26	4,309
576		28	4,352
578		28	6,022
636		28	6,670
665		28	7,294
805		31	7,427
1,000		32	8,094
1,998		32	9,309
2,069		35	11,169
2,433		35	
2,518		37	
2,548		42	
2,726		44	
2,727		46	
2,780		47	
3,275		48	
3,405		51	
3,448		54	
3,805		55	
3,829		59	
3,866		68	
3,960		72	
4,026		80	
4,321		84	
4,359		97	
4,445		101	
4,446		101	
4,665		102	
5,121		105	
Number points examined..	69	Number tubes examined....	84
Total bacteria..............	238,626	Total bacteria...............	88,879
Average per point..........	3,458	Average per tube	1,058

SERIES III.

The following tables give the results of our studies made during the winter, November and December, 1902.

Note especially how vastly superior the glycerinated virus in this series is to Series I and II. Eighty-nine capillary tubes examined averaged only 29 bacteria per tube, whereas in Series II, made in April and May, 1902, the average was 1,058, and in Series I, made in the winter 1901–2, it was 4,698.

MANUFACTURER NO. 1.

Number of organisms found.	
Dry points.	Glycerinated virus.
0	1
0	2
1	2 (Coc. 3.)
1	2
2	3
3 (Coc. 1.)	3
3	4
3	4
5	5
11 (Coc. 2.)	9 (Coc. 4.)

These points were labeled by the manufacturer to be exchanged after November 23, 1902, and were counted November 21, 1902.

The glycerinated lymph was labeled good to December 10, 1902, and was counted November 24, 1902.

The following organisms, some of which resemble the pyogenic cocci, were isolated:

Coccus No. 1.—This staphylococcus was one of 3 colonies which grew on our agar plates planted from a dry point of manufacturer No. 1.

The staphylococcus does not stain by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; does not liquefy gelatine; bouillon is slightly clouded with some white precipitate; on agar, a grayish white moist glistening growth; on potato, white growth, slowly spreading; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth at bend of tube.

Coccus No. 2.—This staphylococcus was one of 11 colonies which grew on our agar plates planted from a dry point of manufacturer No. 1.

The staphylococcus does not stain by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; does not liquefy gelatine; bouillon is clear, with a yellow precipitate; on agar a lemon yellow, moist, shiny growth;

on potato, a scant yellowish growth; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth at bend of tube.

Coccus No. 3.—This staphylococcus was one of 2 colonies which grew on our agar plates planted from a tube of glycerinated virus of manufacturer No. 1.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; in litmus milk, a slight alkaline production; does not liquefy gelatine; bouillon is clear, some white precipitate; on agar, a heavy, glistening pink pigment growth; on potato, very scanty pink growth; in glucose bouillon, no fermentation, slight growth at bend of tube; in lactose bouillon, no fermentation, slight growth at bend of tube.

Coccus No. 4.—This staphylococcus was one of 9 colonies which grew on our agar plates planted from a tube of glycerinated virus of manufacturer No. 1.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; in litmus milk, no change, but a white precipitate; does not liquefy gelatine; in bouillon, clear, white precipitate; on agar, a scanty white, glistening growth; on potato, very scanty white growth; in glucose bouillon, no fermentation, slight growth at bend of tube; in lactose bouillon, no fermentation, slight growth at bend of tube.

MANUFACTURER NO. 3.

Number of organisms found.	
Dry points.	Glycerinated virus.
7,465	41
8,702 (Coc. 5.)	70
10,022	87
10,265	95 (Coc. 9.)
10,982	112 (Coc. 10.)
12,923 (Coc. 6.)	152
15,552	230
25,574	234
26,411 (Coc. 7.)	239
31,380 (Coc. 8.)	

The dry points were labeled to be good until December 7, 1902, and were planted for study November 8, 1902.

The glycerinated lymph was labeled good until December 17, 1902, and was planted November 25, 1902.

The following organisms resembling the pyogenic cocci were isolated:

Coccus No. 5.—This staphylococcus was one of 8,702 colonies which grew on our agar plates planted from a dry point of manufacturer No. 3. The staphylococcus does not stain by Gram's method; does not produce indol in a 24-hour bouillon culture; in litmus milk, slightly acid production; does not liquefy gelatine; bouillon very cloudy, white pellicle, and precipitation; on agar, grayish-white, shiny, moist growth; on potato, heavy yellowish-white growth all over potato; in glucose bouillon, slight fermentation, cloudy all through; in lactose bouillon, slight fermentation, cloudy all through.

Coccus No. 6.—This staphylococcus was one of 12,923 colonies which grew on our agar plates planted from a dry point of manufacturer No. 3. The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; slight acid produced in litmus milk; liquefies gelatine very slowly; bouillon is slightly cloudy, with some white precipitation; on agar, a grayish-white, shiny, moist growth;

on potato, a white, granular, spreading growth; in glucose bouillon, no fermentation, slightly cloudy at bend of tube; in lactose bouillon, no fermentation, slightly cloudy at bend of tube.

Coccus No. 7.—This staphylococcus was one of 26,411 colonies which grew on our agar plates planted from a dry point of manufacturer No. 3. The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; rapidly liquefies gelatine; bouillon is clouded with white precipitation; on agar there is a white, moist, shiny growth; on potato, a white, granular growth, nearly invisible; in glucose bouillon, no fermentation, but growth at bend of tube; in lactose bouillon, no fermentation, but growth at bend of tube.

Coccus No. 8.—This staphylococcus was one of 31,380 colonies which grew on our agar plates planted from a dry point of manufacturer No. 3. The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; liquefies gelatine very slowly; bouillon is slightly cloudy, with white precipitation; on agar, a yellowish-white, moist, shiny growth; on potato, white, granular growth nearly invisible; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth at bend of tube.

Coccus No. 9.—This staphylococcus was one of 95 colonies which grew on our agar plates planted from a tube of manufacturer No. 3. The staphylococcus does not stain by Gram's method; does not produce indol in a 24-hour bouillon culture; strong acid production in litmus milk; does not liquefy gelatine; bouillon is very cloudy, white precipitate; on agar, a grayish-white, moist, shiny, glistening growth; on potato, very heavy, white growth all over; in glucose bouillon, fermentation 1½ inch, growth all through; in lactose bouillon, fermentation three-fourths inch, growth all through.

Coccus No. 10.—This staphylococcus was one of 112 colonies which grew on our agar plates planted from a tube of manufacturer No. 3. The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; does not liquefy gelatine; in bouillon, clear yellowish pellicle and precipitate; on agar, yellow, dry, and granular growth; on potato, very scanty, yellow growth; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth at bend of tube.

MANUFACTURER NO. 4.

Number of organisms found.	
Dry points.	Glycerinated virus.
243	3
262	4
420	6
424	11 (Coc. 13, 14.)
452	16
465	19
475 (Coc. 11, 12.)	31
479	37
526	77 (Coc. 15.)
576	113

The label of this manufacturer stated that the dry points were good till November 28, 1902. They were planted for counting November 18, 1902.

The glycerinated virus was labeled to be good till January 4, 1903, and was planted for study November 28, 1902.

The following organisms resembling the pyogenic cocci were isolated:

Coccus No. 11.—This staphylococcus was one of 475 colonies which grew on our agar plates planted from a dry point of manufacturer No. 4.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; strong acid production in litmus milk; rapidly liquifies gelatine; bouillon is cloudy, with some precipitate; on agar there is a whitish-yellow, moist, shiny growth; on potato, a yellow growth, spreading slowly; in glucose bouillon, no fermentation, but cloudy all through; in lactose bouillon, no fermentation, cloudy all through.

Coccus No. 12.—This staphylococcus was one of 475 colonies which grew on our agar plates planted from a dry point of manufacturer No. 4.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; in litmus milk, acid production, coagulation and casein precipitate; bouillon is slightly cloudy, white precipitate; on agar, whitish gray, moist, glistening growth; on potato, white granular growth; in glucose bouillon, no fermentation, growth all through; in lactose bouillon, no fermentation, growth all through.

Coccus No. 13.—This staphylococcus was one of 11 colonies which grew on our agar plates planted from a tube of manufacturer No. 4.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; does not liquefy gelatine; bouillon slightly clouded, yellow precipitate; on agar, growth transparent, thin layer, no pigmentation; on potato, scanty yellow growth; in glucose bouillon, no fermentation, slight growth at bend of tube; in lactose bouillon, no fermentation, slight growth at bend of tube.

Coccus No. 14.—This staphylococcus was one of 11 colonies which grew on our agar plates planted from a tube of manufacturer No. 4.

The staphylococcus does not stain by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; does not liquefy gelatine; bouillon slightly cloudy, some white precipitate; on agar, a lemon-colored, moist, glistening growth; on potato, no growth; in glucose bouillon, no fermentation, but growth all through; in lactose bouillon, no fermentation, but growth all through.

Coccus No. 15.—This staphylococcus was one of 77 colonies which grew on our agar plates planted from a tube of manufacturer No. 4.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; does not liquefy gelatine; bouillon slightly cloudy, with some precipitate; on agar, a slight granular, scanty growth, no pigmentation; on potato, no growth; in glucose bouillon, no fermentation, growth all through; in lactose bouillon, no fermentation, growth all through.

MANUFACTURER NO. 5.

Number of organisms found.	
Dry points.	Glycerinated virus.
604	2
810	2
1,088	2
1,156	2
1,199	2
1,204	3 (Coc. 16.)
1,399	3
1,452	4
2,246	5
2,584	6

These dry points were stated by the manufacturer to be good until December 4, 1902. They were planted for counting November 7, 1902.

The glycerinated lymph was stated by the manufacturer to be good until December 19, 1902, and was counted November 24, 1902.

The following organisms resembling the pyogenic cocci were isolated:

Coccus No. 16.—This staphylococcus was one of 3 colonies which grew on our agar plates planted from a tube of manufacturer No. 5.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; does not liquefy gelatine; bouillon slightly clouded, with some white precipitate; on agar, a whitish gray, moist, shiny growth; on potato, a white spreading growth; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth at bend of tube.

MANUFACTURER NO. 6.

Number of organisms found.	
Dry points.	Glycerinated virus.
	0
	0
	0
	1
	2
	2
	4
	4
	5
	11 (Coc. 17.)

The glycerinated virus was dated August 8, 1902, and was planted December 3, 1902.

The following organisms resembling the pyogenic cocci were isolated:

Coccus No. 17.—This staphylococcus was one of 11 colonies which grew on our agar plates, planted from a tube of manufacturer No. 6.

The staphylococcus stains by Gram's method. Does not produce indol in a 24-hour bouillon culture; produces acid, with yellow pellicle and precipitate in litmus milk; liquefies gelatine very slowly; bouillon clear, with some yellow precipitate; on agar, a heavy yellow-orange growth; on potato, scant yellowish growth; in glucose bouillon, no fermentation, slightly cloudy at bend of tube; in lactose bouillon, no fermentation, slightly cloudy at bend of tube.

MANUFACTURER NO. 7.

Number of organisms found.	
Dry points.	Glycerinated virus.
3	4 (Coc. 19.)
3	4
5	5
5	5
6	6
7	6
21	7
31	7
48	9 (Coc. 20.)
67 (Coc. 18.)	9 (Coc. 21.)

The points were labeled to be good till December 15, 1902, and were planted November 24, 1902.

The glycerinated virus was labeled good till December 15, 1902, and was counted November 22, 1902.

The following organisms resembling the pyogenic cocci were isolated:

Coccus No. 18.—This staphylococcus was one of 67 colonies which grew on our agar plates planted from a dry point of manufacturer No. 7.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; does not liquefy gelatine; bouillon cloudy with white precipitate; on agar, whitish transparent, slightly granular, scanty growth; on potato, brownish white granular growth; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth at bend of tube.

Coccus No. 19.—This staphylococcus was one of 4 colonies which grew on our agar plates planted from a tube of manufacturer No. 7.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; liquefies gelatine; bouillon cloudy, white pellicle and precipitate; on agar, white shiny growth; on potato, white granular growth; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth at bend of tube.

Coccus No. 20.—This staphylococcus was one of 9 colonies which grew on our agar plates planted from a tube of manufacturer No. 7.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; does not liquefy gelatine; bouillon clear with slightly white precipitate; on agar, whitish gray isolated colonies; on potato, no growth; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth at bend of tube.

Coccus No. 21.—This staphylococcus was one of 9 colonies which grew on our agar plates planted from a tube of manufacturer No. 7.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; a slight acid production in litmus milk; slowly liquefies gelatine; bouillon cloudy with some white precipitation; on agar, heavy yellow growth; on potato, very heavy yellow granular growth; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth at bend of tube.

MANUFACTURER NO. 8.

Number of organisms found.	
Dry points.	Glycerinated virus.
853 (Coc. 22.)	8
868	10
887 (Coc. 23.)	11
1,800	12
1,847	13
1,946	14
2,074	16 (Coc. 25.)
2,244	16
2,912	19
3,754 (Coc. 24.)	20

The dry points of this manufacturer have no date. They were planted December 5, 1902.

The glycerinated virus also had no date and was counted December 2, 1902.

The following organisms resembling the pyogenic cocci were isolated:

Coccus No. 22.—This staphylococcus was one of 853 colonies which grew on our agar plates planted from a dry point of manufacturer No. 8.

The staphylococcus does not stain by Gram's method; does not produce indol in a 24-hour bouillon culture; no change in litmus milk; does not liquefy gelatine; bouillon cloudy, with white precipitate; on agar, white, shiny, moist, glistening growth; on potato, yellowish white growth; in glucose bouillon, no fermentation, slight growth at bend of tube; in lactose bouillon, no fermentation, slight growth at bend of tube.

Coccus No. 23.—This staphylococcus was one of 887 colonies which grew on our agar plates planted from a dry point of manufacturer No. 8.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; very slight acid production in litmus milk; liquefies gelatine very slowly; bouillon cloudy, with white precipitate; on agar, dirty white, shiny, moist growth; on potato, yellow spreading growth; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth at bend of tube.

Coccus No. 24.—This staphylococcus was one of 3,754 colonies which grew on our agar plates planted from a dry point of manufacturer No. 8.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; slight acid production in litmus milk; liquefies gelatine; bouillon is cloudy, with white precipitate; on agar there is a white, shiny, moist, glistening growth; on potato, a white granular growth spreading all over the potato; in glucose agar, no fermentation, but growth at bend of tube; in lactose bouillon, no fermentation, but growth at bend of tube.

Coccus No. 25.—This staphylococcus was one of 16 colonies which grew on our agar plates planted from a tube of manufacturer No. 8.

The staphylococcus does not stain by Gram's method; does not produce indol in a 24-hour bouillon culture; very strong acid production in milk; does not liquefy gelatine; bouillon slightly cloudy, with some white precipitate; on agar, a slight granular scanty growth with no pigmentation; on potato, no growth; in glucose bouillon, no fermentation, growth all through; in lactose bouillon, no fermentation, growth at bend of the tube.

MANUFACTURER NO. 9.

Number of organisms found.	
Dry points.	Glycerinated virus.
5,972	7
7,072	10
7,700	13
7,880	25
8,748 (Coc. 26.)	43 (Coc. 29.)
8,748 (Coc. 27.)	47
8,744	70
11,020	81 (Coc. 30.)
12,972 (Coc. 28.)	85
19,872	105

According to the label, the dry points were prepared by the manufacturer October 31, 1902, and were planted November 29, 1902.

The glycerinated virus was not dated. It was counted December 1, 1902.

The following organisms resembling the pyogenic cocci were isolated:

Coccus No. 26.—This staphylococcus was one of 8,748 colonies which grew on our agar plates, planted from a dry point of manufacturer No. 9.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; is slightly acid in litmus milk; does not liquefy gelatine; bouillon cloudy, with yellow precipitate; on agar, an orange moist glistening growth; on potato, heavy yellow growth; in glucose bouillon, no fermentation, growth all through; in lactose bouillon, no fermentation, growth all through.

Coccus No. 27.—This staphylococcus was one of 8,748 colonies which grew on our agar plates, planted from a dry point of manufacturer No. 9.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; slight acid production in litmus milk; does not liquefy gelatine; in bouillon, heavy white pellicle, with precipitate; on agar, an orange-yellow moist glistening growth; on potato, a yellowish granular growth; no fermentation, cloudy all through, in glucose bouillon; and in lactose bouillon there is no fermentation, but the bouillon is cloudy all through.

Coccus No. 28.—This staphylococcus was one of 12,972 colonies which grew on our agar plates, planted from a dry point of manufacturer No. 9.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; in litmus milk there is a strong acid production, coagulation, and a precipitate of casein; rapidly liquefies gelatine; in bouillon, cloudy with yellow precipitate; on agar, an orange moist glistening growth; on potato, a heavy yellow growth; in glucose bouillon, no fermentation, but cloudy all through; in lactose bouillon, no fermentation, cloudy all through.

Coccus No. 29.—This staphylococcus was one of 43 colonies which grew on our agar plates, planted from a tube of manufacturer No. 9.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; in litmus milk, a strong acid production, coagulation, and precipitate of casein; very rapidly liquefies gelatine; bouillon slightly cloudy with yellow precipitate; on agar, an orange-yellow moist glistening growth; on potato, yellowish slowly spreading growth; in glucose bouillon, no fermentation, but growth all through; in lactose bouillon, no fermentation, but growth all through.

Coccus No. 30.—This staphylococcus was one of 81 colonies which grew on our agar plates, planted from a tube of manufacturer No. 9.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; acid production in litmus milk; liquefies gelatine very slowly; in bouillon, slightly cloudy, with yellow precipitate; on agar, a yellowish brown moist glistening growth; on potato, yellowish slowly spreading growth; in glucose bouillon, no fermentation, growth all through; in lactose bouillon, no fermentation, but growth at bend of tube.

MANUFACTURER NO. 10.

Number of organisms found.	
Dry points.	Glycerinated virus.
None examined.	7
	14
	15
	17
	21
	22
	22
	31
	31 (Coc. 31.)
	53

This glycerinated virus was labeled by the manufacturer to be good until January 1, 1903, and was planted December 4, 1902.

The following organisms, resembling the pyogenic cocci, were isolated:

Coccus No. 31.—This staphylococcus was one of 31 colonies which grew on our agar plates planted from a tube of manufacturer No. 10.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; in litmus milk, produces alkali with light yellow precipitate; does not liquefy gelatine; bouillon clear, with some yellow precipitate; on agar, a heavy yellow, shiny growth; on potato, a scanty yellow growth; in glucose bouillon, no fermentation, but slightly cloudy at bend of tube; in lactose bouillon, no fermentation, slightly cloudy at bend of tube.

MANUFACTURER NO. 11.

Number of organisms found.	
Dry points.	Glycerinated virus.
283 (Coc. 32.)	
324	
333 (Coc. 33.)	
684	
728	
3,696	
9,072	
24,360 (Coc. 34.)	
29,145	
41,000	

These points had no date at all. They were planted November 19, 1902.

The following organisms resembling the pyogenic cocci were isolated:

Coccus No. 32.—This staphylococcus was one of 283 colonies which grew on our agar plates, planted from a dry point of manufacturer No. 11.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; in litmus milk there is a slight acid production, coagulation, and a precipitate of casein; rapidly liquefies gelatine; in bouillon, slightly cloudy, with some yellow precipitate; on agar, orange yellow granular growth; on potato, yellow granular spreading growth; in glucose bouillon, no fermentation, growth at bend of tube; in lactose bouillon, no fermentation, growth all through tube.

Coccus No. 33.—This staphylococcus was one of 333 colonies which grew on our agar plates, planted from a dry point of manufacturer No. 11.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; strong acid production in litmus milk; does not liquefy gelatine; in bouillon, slightly cloudy, with some white precipitate; on agar, a white shiny growth; on potato, nearly invisible white growth; in glucose bouillon, no fermentation, slight growth at bend of tube; in lactose bouillon, slight growth at bend of tube.

Coccus No. 34.—This staphylococcus was one of 24.360 colonies which grew on our agar plates, planted from a dry point of manufacturer No. 11.

The staphylococcus stains by Gram's method; does not produce indol in a 24-hour bouillon culture; in litmus milk there is a strong acid production, coagulation, and precipitate of casein; slowly liquefies gelatine; bouillon is cloudy, with heavy white precipitate; on agar, a whitish yellow, shiny moist growth; on potato, yellow growth with white border; in glucose bouillon, no fermentation, growth all through tube; in lactose bouillon, no fermentation, growth all through.

SUMMARY OF SERIES III.

November and December, 1902.

Number of organisms found.				
Dry points.		Glycerinated virus.		
0	1,204	0	9	
0	1,399	0	10	
1	1,452	0	10	
1	1,800	1	11	
2	1,847	1	11	
3	1,946	2	11	
3	2,074	2	12	
3	2,244	2	13	
3	2,246	2	13	
3	2,584	2	14	
5	2,912	2	14	
5	3,696	2	15	
5	3,754	2	16	
6	5,952	2	16	
7	7,465	2	16	
11	7,632	3	17	
21	7,560	3	19	
31	7,980	3	19	
48	8,702	3	20	
67	8,748	3	21	
243	8,748	4	22	
262	9,072	4	22	
283	9,744	4	25	
324	10,022	4	31	
333	10,265	4	31	
420	10,982	4	31	
424	11,020	4	37	
452	12,923	4	41	
465	12,972	5	43	
475	15,552	5	47	
479	19,872	5	53	
526	24,360	5	70	105
576	25,574	5	70	112
604	26,411	6	77	113
684	29,145	6	81	152
728	31,380	6	85	230
810	44,000	6	87	239
853		7	95	236
868		7		
887		7		
1,088		7		
1,156		8		
1,199		9		
		9		

Number points examined..	80
Total bacteria..............	391,603
Average per point.....	4,895

Number tubes examined.....	89
Total bacteria.................	2,592
Average per tube.............	29

DRY POINTS AND GLYCERINATED VIRUS COMPARED FROM A BACTERIOLOGICAL STANDPOINT.

The question is often asked, Is glycerinated virus superior to dry points?

As the object of glycerinating the virus is to eliminate the foreign bacteria, the answer to this question resolves itself almost entirely into a comparative study of the number and kind of organisms found to contaminate this kind of vaccine.

The result of our studies on this point may be summarized as follows:

Series.	Average number of organisms.	
	Dry points.	Glycerinated virus.
Series I, winter, 1901–2	1,809	1,698
Series II, spring, 1902	3,458	1,058
Series III, winter, 1902–3	1,895	29

It is plain that last winter (Series I) the glycerinated virus contained a number of contaminating bacteria equal in amount to the dry points. This winter (Series III) shows a great improvement in the glycerinated virus and demonstrates conclusively that this form of vaccine may be, and should be freer from impurities than the dry points.

Of course the greater freedom from bacterial impurities of the glycerinated virus to the dry points refers only to those dry points made with fresh "lymph." There can be little choice between the dry points made with glycerinated pulp and the capillary tubes containing the same material.

The pyogenic cocci and other bacteria pathogenic for laboratory animals were found both in the glycerinated virus and on the dry points.

EXPERIMENTAL TETANUS IN VACCINE VIRUS.

Can tetanus be found in the vaccine virus bought in open market?

Many tubes containing glycerinated virus and many points of the dry vaccine were examined bacteriologically for tetanus, but we were unable to discover this organism.

This part of the work was done in several ways. Susceptible animals were inoculated and vaccinated with the contents of many tubes and dry points. Sometimes the total contents of 10 or 12 tubes were injected subcutaneously into guinea pigs or mice. In another series the virus from ivory points or capillary tubes was planted in fresh broth and grown anaërobically, and the resulting growth studied for end-spore-forming rods and also inoculated into animals. In no case was an organism or were symptoms resembling tetanus found.

While these results were most gratifying, they must not be taken as conclusive that tetanus may not sometimes contaminate vaccine virus, although from our studies this must be exceedingly rare. Tetanus organism can not grow or produce its toxin either in the glycerinated virus or on the dry points. It would take gross carelessness to contaminate the vaccine with a sufficient number of tetanus spores to carry the disease to those vaccinated.

It is not a matter of surprise that some outbreaks of tetanus have occurred when thousands of open wounds are presented for the reception of this infection so widely distributed in nature. Local outbreaks or small epidemics of tetanus have been known before the days of antiseptics, due to causes so well understood nowadays, and it seems more likely that carelessness in the dressing or handling of the wound, the pernicious use of shields, or faulty technique in performing the operation has introduced the tetanus spores, than that they were contained in the vaccine virus.

Most of the following work on tetanus was done under my direction by Dr. Edward Francis, and it is a pleasure to acknowledge the care and faithfulness with which he carried out all the exacting details.

VIABILITY OF TETANUS IN VACCINE VIRUS.

Can tetanus live and grow in vaccine virus?

In order to answer this question we contaminated vaccinal serum and the glycerinated pulp with tetanus spores. The serum was dried upon ivory points and the glycerinated pulp was conserved in capillary tubes in a manner similar to the methods now used by the manufacturers. From time to time these points and tubes were examined.

For the purposes of these experiments it was thought best to use a pure culture of tetanus for one series of experiments and for another series to use a mixed culture composed of tetanus, a coccus, and a motile, spore-bearing, saprophytic rod, as representing more nearly the natural conditions under which tetanus would be found to gain entrance into vaccine.

The pure culture of tetanus used in these experiments was taken from our laboratory stock culture. It was planted into ordinary bouillon on March 2 and grown anaërobically in a Novy jar at 37° for seven days, when it contained abundant spores.

VIRULENCE OF THE TETANUS CULTURE USED.

In order to determine the virulence of the pure culture of tetanus used it was tested in white mice, with the following results. Each quantity of pure bouillon culture indicated was inoculated into the right side of a mouse.

Mouse.	Quantity of tetanus culture injected on March 9.
	c. c.
1	0.000065
2	.000049
3	.000039
4	.000032
5	.000028
6	.000024
7	.000021

On March 10 the three mice which had received the largest doses showed typical symptoms of tetanus, while the four mice which had received the smallest doses showed no symptoms.

On March 11 the mouse which received the largest dose was dead. The second, third, fourth, fifth, and sixth showed typical symptoms of tetanus, while the seventh, which received the smallest dose, gave no symptoms whatever of tetanus.

On March 12 mice 2 and 3 were dead; 4, 5, 6, and 7 show typical tetanus.

On March 13 mice 4, 5, and 6 were dead; 7 shows typical tetanus.

On March 14 mouse 7 died.

Table showing virulence of the tetanus culture used in these experiments.

Mouse.	March 9.	March 10.	March 11.	March 12.	March 13.	March 14.
	c. c.					
1	0.000065	Typical tetanus.	Dead.			
2	.000049	.....do	Typical tetanus.	Dead.		
3	.000039	.....do	.....do	Do.		
4	.000032	Negative .	.....do	Typical tetanus.	Dead.	
5	.000028	.....do	.....do	.....do	Do.	
6	.000024	.....do	.....do	.....do	Do.	
7	.000021	.....do	Negative .	.....do	Typical tetanus.	Dead.

From these tests we considered 0.000021 c. c. to constitute about the minimum fatal dose of our culture for an average-sized white mouse.

VIABILITY OF A PURE CULTURE OF TETANUS IN GLYCERINATED VACCINE VIRUS.

The vaccine pulp was taken from the heifer on February 15 and subjected to the action of glycerin by the vaccine manufacturer for twenty-five days. It was then, on March 12, obtained by us and contaminated with our pure culture of tetanus in the following manner:

We aimed to imitate as closely as possible the ordinary conditions under which we find glycerinated vaccine on the market. So we drew out in the flame a large number of capillary tubes of the length and caliber ordinarily used by manufacturers to contain material sufficient for one vaccination. Definitely measured amounts of the pure culture of tetanus were added to large drops of the glycerinated virus, and this mixture was drawn up into the small capillary tubes, which were hermetically sealed in the usual manner. In this way we had an accurate gauge of the exact amount of tetanus put into each capillary tube.

These tubes were filled with the glycerinated virus and tetanus spores on March 12. Each capillary tube contained one drop of glycerinated virus plus the following amounts of tetanus culture:

[.000021=MLD, minimal lethal dose.]

First series	=0.000055 c. c.	(about 2½ MLD).
Second series	.00011 c. c.	(about 5 MLD).
Third series	.00022 c. c.	(about 10 MLD).
Fourth series	.00040 c. c.	(about 20 MLD).
Fifth series	.00074 c. c.	(about 40 MLD).
Sixth series	.013 c. c.	(about 500 MLD).

The capillary tubes were tested immediately in mice as a control and then placed in the cool chamber at 20°–22° C., protected from light, and again tested at intervals in mice and in cultures, as indicated in the following tables:

Capillary tubes containing 0.000055 c. c. of tetanus culture added to glycerinated virus, March 12, and tested at intervals as shown.

[P=positive, i. e., symptoms of tetanus; N=negative, i. e., no symptoms; †=death.]

Date prepared.	Inoculated into mice.	Number of days.	Symptoms.								Result.
			1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	
Mar. 12	Mar. 12	0	P	P	P	P	P	†	.....	.....	Positive; death.
Do	Apr. 11	30	P	P	†	.....	.....	.....	.....	.....	Do.
Do	May 21	70	P	P	P	P	†	.....	.....	.....	Do.
Do	June 17	97	N	P	P	P	P	P	P	†	Do.
Do	July 19	129	N	P	P	.....	.....	.....	.....	.....	Positive; recovered.
Do	Aug. 1	142	N	N	P	P	P	.....	.....	.....	Do.
Do	Aug. 28	169	N	.....	.....	.....	.....	.....	.....	.....	Negative.
Do	Sept. 3	175	N	.....	.....	.....	.....	.....	.....	.....	Do.

We see from this series that the tetanus spores neither multiplied nor produced toxin. In fact, they gradually lost virulence. Each capillary tube in this series of experiments contained approximately two and a half times the minimal lethal dose of the tetanus culture. It will be noted that the virulence began to diminish noticeably at the fourth month and failed to produce tetanus after the lapse of five months.

Capillary tubes containing 0.00011 c. c. of tetanus culture added to glycerinated virus, March 12, and tested at intervals as shown.

Date prepared.	Inoculated into mice.	Number of days.	Symptoms.										Result.
			1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	
Mar. 12..	June 17	97	N	P	P	†	....	....	....	....	....	....	Positive; death.
Do...	Aug. 28	169	N	N	N	N	....	....	....	....	....	....	Negative.
Do...	Sept. 3	175	N	N	N	N	....	....	....	....	....	....	Do.
Do...	Sept. 9	181	N	N	N	N	....	....	....	....	....	....	Do.

The series contained approximately five times the minimal fatal dose of tetanus. It was first tested after the lapse of three months, when it was still virulent; but after seven months it had entirely lost its power of producing the disease when inoculated directly into mice. Evidently the tetanus spores did not germinate or produce toxin.

Capillary tubes containing 0.00022 c. c. of tetanus culture added to glycerinated virus, March 12, and tested at intervals as shown.

Date prepared.	Inoculated into mice.	Number of days.	Symptoms.										Result.
			1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	
Mar. 12	Sept. 1	173	N	N	P	P	P	P	P	P	P	P	Positive; recovered.
Do...	Oct. 1	203	N	P	P	P	P	P	....	....	....	....	Do.
Do...	Nov. 15	248	N	P	P	P	P	P	P	....	....	....	Do.
Do...	Jan. 1	295	N	....	....	....	....	....	....	....	....	....	Negative.

This series was contaminated with ten times the minimal fatal dose. In this large quantity the spores maintained their toxicity a very long time—two hundred and forty-eight days—although they failed to produce the disease after ten months.

Capillary tubes containing 0.00040 c. c. of tetanus culture added to glycerinated virus, March 12, and tested at intervals as shown.

Date prepared.	Inoculated into mice.	Number of days.	Symptoms.										Result.
			1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	
Mar. 12	Sept. 1	173	N	P	P	P	P	P	P	P	P	†	Positive; death.
Do...	Oct. 1	203	N	P	P	P	P	P	P	P	P	P	Positive; recovered.
Do...	Nov. 15	248	N	N	P	P	P	P	P	P	P	P	Do.
Do...	Jan. 1	295	N	....	....	....	....	....	....	....	....	....	Negative.

Each capillary tube in this series contained about twenty times the minimal fatal dose of tetanus, which lost its virulence after ten months.

Capillary tubes containing 0.00074 c. c. of tetanus culture added to glycerinated virus, March 12, and tested at intervals as shown.

Date prepared.	Inoculated into mice.	Number of days.	Symptoms.										Result.
			1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	
Mar. 12	Sept. 1	173	P	P	†	.....	.....	.....	.....	.....	.....	.....	Positive: death.
Do...	Oct. 1	203	P	P	P	P	P	P	P	P	P	†	Do.
Do...	Nov. 15	248	P	P	†	.....	.....	.....	.....	.....	.....	.....	Do.
Do...	Jan. 1	295	P	P	†	.....	.....	.....	.....	.....	.....	.....	Do.
Do...	Feb. 1	326	N	P	†	.....	.....	.....	.....	.....	.....	.....	Do.
Do...	Mar. 2	355	N	P	†	.....	.....	.....	.....	.....	.....	.....	Do.

It will be seen that this large amount of tetanus (about forty times the minimal fatal dose) remained alive and virulent in glycerinated virus one year.

Capillary tubes containing 0.013 c. c. of tetanus culture added to glycerinated virus, March 12, and tested at intervals as shown.

Date prepared.	Inoculated into mice.	Number of days.	Symptoms.										Result.
			1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	
Mar. 12	Sept. 1	173	P	†	.....	.....	.....	.....	.....	.....	.....	.....	Positive: death.
Do...	Oct. 1	203	P	†	.....	.....	.....	.....	.....	.....	.....	.....	Do.
Do...	Nov. 15	248	P	†	.....	.....	.....	.....	.....	.....	.....	.....	Do.
Do...	Jan. 1	295	P	†	.....	.....	.....	.....	.....	.....	.....	.....	Do.
Do...	Feb. 1	326	P	†	.....	.....	.....	.....	.....	.....	.....	.....	Do.
Do...	Mar. 2	355	N	P	†	.....	.....	.....	.....	.....	.....	.....	Do.

This large amount (about 500 × MLD) has also remained alive and virulent for mice one year. The material is not yet exhausted, and will be tested from time to time.

VIABILITY OF PURE CULTURE OF TETANUS ON DRY POINTS.

The serum which exuded from the site of the inoculation after the superficial layers of the variolous eruption was removed was obtained from a calf on March 12, 1902. This serum, commonly called "lymph," when dipped or brushed upon the ivory points and allowed to dry, formerly constituted the "dry points" of the manufacturers. At present some dry points are made with glycerinated virus alone or with glycerinated pulp mixed with blood serum and in other ways. We, however, preferred to use the "lymph" or serum, as it seemed to offer a more favorable medium for preserving the vitality of the tetanus spores.

The sterile ivory points were prepared in the usual manner with the vaccinal serum but contaminated with definite measured amounts of tetanus culture.

Each point of the first series contained 0.00012 c. c. of pure bouillon culture of tetanus; each point of the second series contained 0.0002 c. c. of pure bouillon culture of tetanus; each point of the third series contained 0.0004 c. c. of pure bouillon culture of tetanus; each point of the fourth series contained 0.001 c. c. of pure bouillon culture of tetanus.

These were allowed to dry in the usual manner and kept in the cool chamber at 20°–22° C., well protected from the light. These points were tested at intervals on mice and in cultures as shown in the following tables:

Dry points containing vaccine serum ("lymph") were contaminated with 0.00012 c. c. of tetanus culture and kept at 22° C. in the dark. From time to time they were tested, with the following result:

Date prepared.	Inoculated into mice.	Number of days.	Symptoms.										Result.
			1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	
Mar. 12	May 28	77	N										Negative.
Do	June 17	107	N										Do.
Do	June 27	117	N										Do.

It will be seen that a quantity of tetanus spores equal to five times the minimal lethal dose lost its toxicity for mice within seventy-seven days. The spores, however, were not dead, for these points planted in bouillon and grown anaerobically in a Novy jar for one week gave a culture rich in end spore-bearing rods, which caused typical tetanus in a mouse in doses of 0.001 c. c.

Dry points containing vaccine serum ("lymph") were contaminated with 0.0002 c. c. of tetanus culture and kept at 22° C. in the dark. From time to time they were tested, with the following result:

Date prepared.	Inoculated into mice.	Number of days.	Symptoms.										Result.
			1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	
Mar. 12	May 28	77	N										Negative.
Do	June 17	107	N										Do.
Do	June 27	117	N										Do.

These points, containing approximately ten times the minimal fatal dose of tetanus, apparently soon lost their virulence when inoculated directly into mice. But when grown anaerobically, as above, the spores multiplied and regained their pathogenic power.

Dry points containing vaccine serum ("lymph") were contaminated with 0.0004 c. c. of tetanus culture and kept at 22° C. in the dark. From time to time they were tested, with the following result:

Date prepared.	Inoculated into mice.	Number of days.	Symptoms.										Result.
			1st. day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	
Mar. 12	May 28	77	P	P	P	P	P	P	P	P	P	P	Positive; recovered.
Do...	June 17	107	N	.....	.....	.....	.....	.....	.....	.....	.....	.....	Negative.
Do...	June 27	117	N	.....	.....	.....	.....	.....	.....	.....	.....	.....	Do.

This series of dry points, contaminated with about forty times the minimal dose of tetanus, remained virulent over seventy-seven days, but failed to produce symptoms after one hundred and seven days. The spores, however, were not dead; for, planted in bouillon and grown in a Novy jar for seven days, they gave a rich culture, which produced typical symptoms of tetanus in a mouse.

Dry points containing vaccine serum ("lymph") were contaminated with 0.001 c. c. of tetanus culture. From time to time they were tested, with the following result:

Date prepared.	Inoculated into mice.	Number of days.	Symptoms.										Result.
			1st. day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	
Mar. 12..	May 28	77	P	†	.....	.....	.....	.....	.....	.....	.....	.....	Positive; death.
Do.....	June 27	107	P	P	†	.....	.....	.....	.....	.....	.....	.....	Do.
Do.....	Aug. 1	142	?	P	P	P	P	†	.....	.....	.....	.....	Do.
Do.....	Aug. 28	169	N	P	†	.....	.....	.....	.....	.....	.....	.....	Do.
Do.....	Sept. 30	202	N	P	.....	.....	.....	.....	.....	.....	.....	.....	Positive.
Do.....	Nov. 15	248	P	P	P	P	P	P	P	P	P	†	Positive; death.
Do.....	Jan. 1	295	N	N	P	P	P	P	P	P	†	.....	Do.
Do.....	Feb. 1	336	N	.....	.....	.....	.....	.....	.....	.....	.....	.....	Negative.

The large amount of tetanus in this series of points remained virulent, when directly inoculated into mice, for a period of two hundred and ninety-five days. This shows well the long life of dried tetanus spores when kept in a cool place, protected from the light—that is, under the conditions under which vaccine virus is usually conserved.

VIABILITY OF TETANUS IN MIXED CULTURE IN GLYCERINATED VIRUS.

In the following experiments we used very small quantities of a culture of tetanus mixed with other organisms, viz, a coccus and a saprophytic motile rod with a central spore. This mixture was planted into ordinary bouillon April 1 and incubated at 37° C. in a Novy jar for two weeks. The resulting growth was then tested on mice to determine its virulence. Each of two mice were given 0.00002 of the culture. Both animals took the disease. The one died in eight days; the other recovered. One month later, viz. May 28, two more mice were inoculated with an equal amount of the same culture, and both died. This amount was, therefore, considered to represent approximately the minimal fatal dose, and was used to contaminate the vaccine virus.

The glycerinated virus of two manufacturers was bought in the open market. With the mixed tetanus culture we prepared an artificial contamination of both these viruses and sealed them hermetically in small capillary tubes. By means of dilution each capillary tube was made to contain just 0.00002 c. c. of the tetanus culture in addition to a large-sized drop of the glycerinated virus.

These tubes were kept at 20°–22° C. in a dark place and tested from time to time.

Glycerinated virus (manufacturer No. A) contaminated with 0.00002 c. c. of a mixed culture of tetanus.

Date prepared.	Inoculated into mice.	Number of days.	Symptoms.										Result.
			1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	
Apr. 30	Apr. 30	0	P	P	P	P	P	P	P	P	P	P	Positive; recovered.
Do..	May 28	28	N										Negative.
Do..	June 17	48	N										Do.
Do..	June 27	58	N										Do.

We see from this table that one month's action of the glycerinated virus reduced the virulence of the tetanus which it contained to a point where it no longer produced any symptoms on mice.

A comparison of the table above with the control which immediately precedes it indicates strikingly the attenuating power of the glycerinated virus in this instance. Observe that on May 28 the glycerinated tube produced no symptoms of tetanus, while the two control tests made on the same date both proved typical tetanus. Whether the virus had killed the tetanus or merely diminished its virulence was determined by planting on July 8 six of the tubes each into a separate cubic centimeter of bouillon and growing it under anaërobic conditions for one week. At the end of this time we tested the growth in mice and were surprised to find that three of the tubes when injected into mice caused no sickness whatever, while the other three tubes produced typical symptoms of tetanus in mice.

We conclude that the glycerinated virus killed the tetanus entirely in some of the tubes in two months and that if it had been allowed to act longer on the other tubes they, too, probably would have contained no living tetanus.

Glycerinated virus (manufacturer No. B) contaminated with 0.0002 c. c. of a mixed culture of tetanus.

Date prepared.	Inoculated into mice.	Number of days.	Symptoms.										Result.
			1st day.	2d day.	3d day.	4th day.	5th day.	6th day.	7th day.	8th day.	9th day.	10th day.	
Apr. 30..	Apr. 30	0	P	P	P	P	(†)	.....	.....	.....	.....	.....	Positive; death.
Do.....	May 28	28	P	P	P	P	P	P	P	P	P	P	Positive; recovered.
Do.....	June 17	48	N	P	P	P	P	P	P	(†)	.....	.....	Positive; death.
Do.....	June 27	58	N	P	P	P	P	P	P	P	P	(†)	Do.
Do.....	...do....	58	N	N	N	P	P	P	P	P	P	P	Positive; recovered.
Do.....	July 18	79	N	N	P	P	P	P	P	P	P	P	Do.
Do.....	Aug. 1	93	N	N	P	P	P	P	P	P	P	P	Do.
Do.....	Aug. 28	120	N	.....	.....	.....	.....	.....	.....	.....	.....	.....	Negative.
Do.....	Sept. 3	126	N	.....	.....	.....	.....	.....	.....	.....	.....	.....	Do.
Do.....	Sept. 6	129	N	.....	.....	.....	.....	.....	.....	.....	.....	.....	Do.
Do.....	Sept. 29	152	N	.....	.....	.....	.....	.....	.....	.....	.....	.....	Do.

This table shows that after August 28 the tubes failed to produce tetanus in the mice. Therefore, on September 30, we subjected the remaining two tubes to cultivation in bouillon for one week and found that in one of the tubes the tetanus was still alive, for the growth showed rods bearing end spores and produced typical tetanus in mice. In the other tubes there was no growth of tetanus.

A comparison of these two tables shows that make No. B of glycerinated virus required four months to attenuate the same amount of tetanus which make No. A attenuated in one month and killed completely in two months. This fact led to a study of the comparative effects of the different percentages of pure glycerin on the tetanus organism and its toxin. This study will be reported upon later.

VIABILITY OF TETANUS IN MIXED CULTURE ON DRY POINTS.

The same mixed culture of tetanus used in the two preceding experiments was also used to contaminate dry points. The tips of the sterilized ivory points were dipped into vaccine serum freshly obtained from the calf. To each point was then immediately added a definite measured amount of the tetanus culture. The points were allowed to dry in the air and kept in a cool (20°–22° C.), dark place until tested.

To contaminate the first series 0.001 c. c. of the tetanus culture was used; 0.0004 c. c. of the tetanus culture was used to contaminate the second series; 0.0002 c. c. of the tetanus culture was used to contaminate the third series; 0.00012 c. c. of the tetanus culture was used to contaminate the fourth series.

Series of dry points containing various amounts of mixed culture of tetanus, prepared June 2.

Amount of tetanus culture.	Inoculated into mice.	Result.
c. c.		
0.001	June 17..........	Remained negative.
	June 27..........	Do.
.0004	June 17..........	Do.
	June 27..........	Do.
.0002	June 17..........	Do.
	June 27..........	Do.
.00012	June 17..........	Do.
	June 27..........	Do.

Compare this table with the control experiments on page 28 and we see the striking attenuating effect of exposure of tetanus on dry points.

For instance: On May 28 two tests of the control culture killed mice with typical symptoms of tetanus; whereas, on June 17, after an exposure of two weeks on dry points, doses of tetanus which represented as much as fifty times the control doses had been rendered nontoxic for mice. It now remained to determine whether the tetanus on the point was dead or only attenuated. It was found after growing a point of each series in 1 c. c. of bouillon for one week that the growth showed end-spore bearing organisms in microscopic preparations and produced typical symptoms of tetanus when inoculated into mice.

Again, on October 4, four months after the dry points were prepared, specimens from series 0.0004, 0.0002, and 0.00012 c. c. were planted into bouillon and grown anaërobically. The culture again contained end spore bearing organisms which, inoculated into mice, produced typical symptoms of tetanus. The tetanus spores on the points were therefore not dead, although they had lost their virulence for mice when inoculated directly.

SUMMARY AND CONCLUSIONS.

We examined the vaccines of 10 different manufacturers during a period of more than a year.

All the samples examined were purchased on the open market, care being taken to buy unbroken original packages from reliable pharmacists who keep the product under proper conditions of light and temperature. With a few exceptions, the vaccine was examined before the time limit expired, if the time was given by the manufacturer.

Of 190 dry points examined we found an average of 4,354 bacteria per point. A number of these points contained over 15,000 and one as high as 44,000 organisms.

Of 244 tubes of glycerinated virus examined we found an average of 1,742 bacteria per tube. A number of these capillary tubes contained over 10,000 bacteria and one as high as 30,000. This is of course much more than a carefully prepared glycerinated virus should contain.

We found the pus cocci and other bacteria, pathogenic for laboratory animals, in both the dry points and the glycerinated virus.

We have demonstrated that some of the glycerinated virus marketed during the winter of 1901-2 contained an excessive number of bacteria, which decreased notably after a few weeks, indicating the sale of a "green" or unripe product; that is to say, the virus was not glycerinated a sufficient length of time before it was sold.

It was also plain from our studies that too much confidence was placed by the producers in the germicidal power of glycerin.

The excessive impurities found in some of the glycerinated virus upon the market we believe was largely due to this overconfidence in the germicidal value of glycerin; operators become careless, trusting to the glycerin to purify their product. We know that glycerin is too feeble in its properties to purify vaccine matter which has initial contamination such as our work indicates.

After we called attention to this point we found a marked improvement in the glycerinated virus on the market. For instance:

During the winter of 1901-2 the glycerinated virus contained an average of 4,698 bacteria per tube.

In the spring (April and May) of 1902 the average fell to 1,058 bacteria per tube.

This winter (November and December), 1902, the average of 89 tubes examined was only 29 bacteria—maximum 239.

Glycerinated virus, when properly prepared and kept a sufficient length of time, is freer from impurities than dry points made with fresh "lymph."

There is practically no difference between the glycerinated virus dried upon ivory points and that hermetically sealed in capillary tubes, so far as bacteriological impurities are concerned.

It will be seen that there is practically no vaccine on the market free from bacterial contamination, although the product of some manufacturers is remarkably pure.

The unevenness of the purity of vaccine as marketed by some manufacturers is very marked. The greater contamination of the vaccine during the winter of 1901-2 may be accounted for, in part, by the undue haste and accompanying carelessness incidental to supplying the increased demand for vaccine virus at that period.

We have examined a great number of dry points and the contents of glycerinated tubes for tetanus, but have been unable to find the organism of this disease.

Tetanus spores may live a long time in vaccine virus. We have found them alive and virulent on dry points after two hundred and ninety-five days and in glycerinated virus sealed in capillary tubes three hundred and fifty-five days.

Tetanus may become a contaminating element of vaccine before it leaves the heifer. During the period of three to five days which elapses between the vaccination of the heifer and the removal of the virus there is opportunity for tetanus to find a lodgment in the eruption on the heifer's body surface, provided tetanus is present in the stall or stable surroundings of the animal.

If tetanus reaches the heifer's vaccinated area it may contaminate both the "dry points," which are made directly from the "lymph," and also the vaccine pulp, which the manufacturer subjects for a longer or shorter time to the germicidal action of glycerin before he markets it as "glycerinated virus."

Tetanus added to glycerinated vaccine virus does not germinate when kept hermetically sealed and under anaërobic conditions in small capillary tubes. It gradually loses its virulence both in the tubes and on the ivory points. Although the virulence is lost, the tetanus spores are not necessarily dead; for, while they will not produce symptoms when inoculated directly into mice, they may be revived into active virulent cultures by growing in fresh bouillon under favorable conditions. That is to say, the vegetability of the spore remains active long after it has lost its power to produce the disease when inoculated directly into mice.

Therefore, in looking for tetanus in vaccine virus it is best to make cultures first and study the growth for end spore-bearing rods and then test the effects of the culture in animals.

The spores of tetanus lose their virulence and die much more quickly in the vaccine lymph on dry points than in the glycerinated tubes.

The vitality of tetanus in glycerinated virus depends largely upon the number of spores contaminating the virus. Large quantities, namely, forty times the minimal lethal dose (40 × MLD), remain alive and active over a year. Smaller amounts may disappear in four to seven months. Very small amounts (1 × MLD) have in one of our experiments lost virulence in one month and failed to grow in bouillon in two months.

On the other hand, these very small amounts of tetanus may remain active for months in glycerinated virus in capillary tubes. In one instance as small a quantity as 0.000055 c. c., which was about two and a half times the minimal fatal dose, remained active seven months.

In vaccinal "lymph" on dry points the spores may begin to lose their virulence in two weeks and be dead in two months. Usually they live longer. We found them alive and virulent in points that had been contaminated with 50 × MLD about ten months (two hundred and ninety-five days).